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Crabgrass and witchgrass susceptibility to simazine and atrazine.

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CRABGRASS AND WITCHGRASS SUSCEPTIBILITY TO SIMAZINE AND ATRAZINE

A Dissertation Presented

By

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Plant and Soil Sciences

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I N T R O D U C T I O N

The rapid advancement in weed technology over the past two decades has led to the development of several new and effective herbicides which were required to meet the expanding agricultural demands of an ever-increasing world population. Environmental concern has led people to question the practice of applying chemicals to crops and soil so it is necessary to study the metabolism and breakdown of each chemical and determine if it is a potential hazard to the environment. In response to these needs a number of investigations have been undertaken to determine the decomposition and metabolism of herbicidal compounds. These investigations have not only given us new insights into the complex biochemistry of plants, animals and microbial systems, but have also provided us with more useful scientific rationale for the synthesis of more effective herbicides.

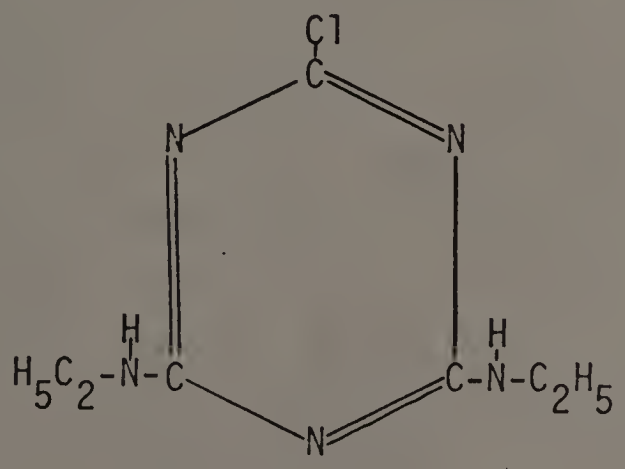
The high biological activity of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) and simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) for control of a wide spectrum of plants has made them extremely useful as selective herbicides. These substituted chlorotriazines are two of the most widely used herbicides for the control of annual weeds in field corn.

Witchgrass (Panicum capillare L.) is a common weed in the Northeastern United States, but is not a serious problem as it appears to be susceptible to both simazine and atrazine at recommended rates (70). On the other hand, reports on the success of large crabgrass (Digitaria sanguinalis (L.) Scop.) control by simazine and atrazine are inconclusive (4,50,57,74,77,103) indicating great variability in experimental conditions (especially soil type) and/or the possibility that different strains exhibit varying tolerances to the chloro-s-triazines (99). However, it appears that large crabgrass is more tolerant to the chloro-s-triazine herbicides than other annual weeds commonly found in corn. Witchgrass on the other hand appears to be susceptible to both simazine and atrazine at recommended rates (70).

Preliminary work in this investigation revealed two important findings: (1) Simazine was more toxic than atrazine to both witchgrass and crabgrass and (2) witchgrass was more susceptible than crabgrass to both chloro-s-triazines. This investigation was undertaken to determine the factors underlying the differential toxicity of simazine and atrazine to crabgrass and witchgrass, and the greater susceptibility of witchgrass as compared to crabgrass to both chloro-s-triazines.

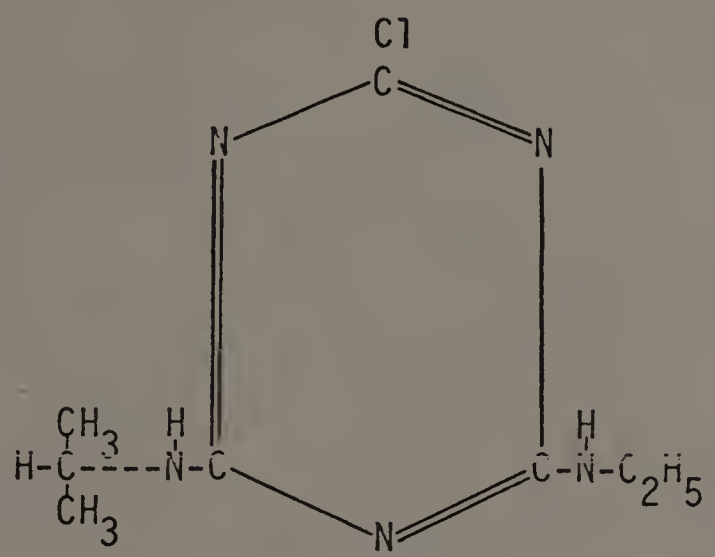
R E V I E W O F L I T E R A T U R E

Chemical nature of the s-triazine Herbicides: Simazine and atrazine belong to a group of heterocyclic compounds known as the substituted triazines. The ring structure of the s-triazine molecule is 6-membered, and contains 3 nitrogen atoms. The selectivity of these herbicides is markedly influenced by the substituent groups attached to the s-triazine nucleus (39). Most of the s-triazines showing herbicidal activity have alkyl-substituted amino groups in the four and six positions of the ring. In the simazine molecule, both of these positions are occupied by ethyl amino groups and accordingly the organic nomenclature of simazine is 2-chloro-4,6-bis(ethylamino)s-triazine.



SIMAZINE

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) structurally differs from simazine only in the substitution of an isopropylamino group for an ethylamino group at the six position.



ATRAZINE

The mode of action of both of these herbicides is the same. They do not prevent germination of weed seeds, but destroy weed seedlings shortly after emergence. These compounds have been shown to be effective inhibitors of the Hill reaction in photosynthesis (34, 65), and are known to reduce the rate of CO_2 fixation in plants (7, 102, 109). Gast (35) showed that in Coleus Blumei simazine blocked starch production in the light and that this effect was overcome by the addition of sucrose to starch-free leaves.

Other studies by Singh and West (83) demonstrated an alteration of chloroplast protein of oat plants by simazine. They also noted marked differences in the amino acid incorporation ability of the chloroplasts.

Cuticular penetration of simazine is limited (51). Simazine is most effective when applied to the soil before weeds emerge. It enters plants primarily through the roots and its effect is dependent upon irrigation, rainfall or cultivation to move it to the root zone. The herbicide is translocated through the xylem in the transpiration stream and tends to accumulate around the margins of dicoteledonous leaves and in the tips of monocoteledonous leaves. Because of the small amount taken up by the foliage, simazine is not used as a post-emergence spray (51).

Atrazine produces similar pre-emergence effects on plants. However, it differs from simazine in one important respect. Atrazine is absorbed through the foliage of plants more readily than simazine. Atrazine is therefore also used as an early post-emergence herbicide.

The s-triazine molecule exhibits few characteristics of an aromatic nucleus. This is due to the presence of the strong electronegative N atoms in the ring. Much of the chemistry of the s-triazine compounds is simply the chemistry

of the substituent groups attached to the ring which is very often not involved in the reaction (105).

Non-biological Detoxication of s-triazine Herbicides

Research on non-biological detoxication has been for the most part centered on volatilization, soil adsorption, photodecomposition, hydroxylation and dealkylation.

Volatilization

Volatilization has been implicated in the loss of herbicidal s-triazine from the soil. The rate of loss by this process is related to soil temperature, moisture and the chemical nature of the s-triazine. Davis et al. (20) noted a 50 percent loss of simazine by volatilization from a metal surface at 160° to 165°F. They reported less volatilization at lower temperatures. Other workers have reported the loss of s-triazines by volatilization (23, 28, 48).

Kearney et al. (49) compared the rate of volatility of s-triazine herbicides from moist and dry soils. They reported that simazine appeared to be more volatile from dry soil whereas atrazine volatility was greater from wet soil. They noted that soil type influenced vapor loss of the s-triazine herbicides but had less effect on simazine than on atrazine. They also found that volatility from nickel plated planchets of the seven compounds investigated varied directly with the vapor pressure of the herbicides.

Soil Adsorption

The adsorption - desorption of pesticides by soil systems is directly influenced by soil reaction, colloid or soil type, physical-chemical nature of the pesticide, temperature, and the formulation of the pesticide (8).

The adsorption of herbicides (of widely different molecular structures) was found by Frissel and Bolt (19) to increase as the pH of the soil solution decreased. Bailey et al. (10) noted that adsorption occurred to a much greater extent (regardless of the chemical nature of the adsorbate) on the highly acid-hydrogen-montmorillonite (pH 3.35) than on the near neutral sodium-montmorillonite (pH 6.8). In an adsorption study of 13 s-triazines by montmorillonite, Weber (104) reported that maximum adsorption of all the compounds occurred at a pH close to the dissociation constant of each compound. Some desorption of the adsorbed triazines occurred when the pH was lowered further. This work is not in agreement with earlier investigations by Frissel and Bolt (32), who found an increase in adsorption as the pH was decreased from about three to four pH units above the dissociation constant down to a pH of one.

The nature of the colloid also influences the adsorption-desorption phenomenon of the s-triazines. The extent to which these processes occur are directly related to the configura-

tion and the area of the surface, and to the magnitude, distribution and intensity of the electrical field at the surface (9). The 1:1 clay minerals, primarily those of the kaolinite group, because of their low cation exchange capacity and low surface area, have very limited adsorption capacities. The 2:1 minerals such as montmorillonite and vermiculite on the other hand, have considerably higher adsorptive capacities.

The physical-chemical nature of the adsorbate reflects its adsorptive properties. Of major importance are the chemical character, shape and configuration, water solubility, acidity or basicity of the molecule, molecular size and polarity. Little work has been done to date to determine the relative importance of each of these factors. Bailey and White (10) studied the effect of the functional group and the nature and position of ring substitution on the magnitude of adsorption of a variety of compounds. They concluded that the chemical nature of the molecule influenced adsorption on to a colloidal system in different ways: (1) affects the water solubility of the molecule and (2) determines if the molecule is fundamentally acidic or basic.

Within a family of compounds there appears to be a relationship between water solubility and the extent of adsorption. Bailey *et al*. (9) compared the extent of adsorption of a number of herbicides on to sodium and hydrogen mont-

morillonite. They discovered that a direct relationship existed between the water solubility and adsorbability for the s-triazines on Na-montmorillonite. This relationship between water solubility and adsorbability was confirmed by Talbert and Fletchall (96) who noted that there was greater adsorption of methoxy- and methylmercapto-derivatives than there was of the chloro-s-triazines.

Exchange reactions tend to be temperature dependent. Adsorption processes are exothermic and desorption processes are endothermic. An increase in temperature tends to reduce adsorption and increase the desorption process. However, Harris and Warren (45) noted that the adsorption of simazine, atrazine and monuron by bentonite was greater at 0° than at 50°C.

An indirect influence of temperature on adsorption is through its effects on vapor pressure and solubility. In general increased temperature leads to decreased adsorption. However, Freed et al. (31) noted increased adsorption of EPTC at higher temperatures, which very likely was a reflection of the effect of temperature on solubility.

Photodecomposition

Little critical research has been done to determine actual loss from photodecomposition of the s-triazines. However, sufficient research has been carried out to demon-

strate that some loss due to photodecomposition of these herbicides does occur.

Dewey (25) reported a decrease in simazine activity after the compound was irradiated with a mercury-vapor high-pressure lamp. Inactivation of simazine by sunlight was demonstrated by Sheets and Danielson (79). Mitchell (60) irradiated simazine on filter paper with U V light, but his results were inconclusive. Comes and Timmons (19) demonstrated that atrazine and simazine on the soil surface may be detoxified by sunlight. Atrazine loss in the spring from irradiation, as determined by oat bioassay, was 47 percent in 25 days and 73 percent in 60 days, while loss in dark cultures was negligible. In the summer, 65 to 80 percent of the atrazine applied to the soil surface was detoxified. In the latter experiment, summer soil temperatures ranged from 150° to 180°F, and volatilization of the herbicides may have occurred, making it difficult to measure the extent of photodecomposition. The simazine loss reported in this experiment, was in the order of 25 percent in the spring over a 25 day period.

Hydroxylation Reactions

The hydrolysis of s-triazines in soil to non-phytotoxic hydroxy analogs has been reported (1,5,6,43,84). A number of factors affect the rate of hydroxylation of s-triazines

in soils. Armstrong et al. (6) showed that soil pH and organic matter content largely control the rate of atrazine hydroxylation. Atrazine hydroxylation rates were greatest in soils high in organic matter and low pH. Only a very slight effect on degradation rate was obtained by increasing the clay content of soils used in the study. Working with soils of high organic matter content and high clay content, Harris (43) noted that montmorillonite protected simazine from hydrolysis through adsorption, while organic matter increased the degradation rate by acting as a catalytic agent.

Armstrong et al. (6) demonstrated that chemical hydrolysis of atrazine occurred in strongly basic or acid solutions. These workers postulated that acid hydrolysis results from protonation of a ring or chain nitrogen atom followed by cleavage of the C-Cl bond by water. The ring C-atom bonded to the Cl group, surrounded by electronegative Cl and N atoms, is electron deficient and therefore susceptible to displacement by strong nucleophilic agents such as OH ions and hence its susceptibility to alkaline hydrolysis.

Dealkylation Reactions

Plimmer et al. (68) carried out extensive studies on the N-dealkylation of s-triazines by soil fungi, higher plants and soil. They reported that free radical reactions caused

N-dealkylation of s-triazines and suggested that a similar mechanism occurred in plants and soil fungi.

Persistence of Triazine Herbicides in Soil

The s-triazines exhibit varying degrees of persistence in soils. In general persistence is related to chemical structure (81). Triazine herbicides containing methoxy substituents on the two position of the ring are generally more persistent than those containing methylthio- or chloro- substituents (14,80,82,94). Differences in persistence have been reported among the 2-chloro-s-triazines. Horowitz (47) in field experiments in Israel noted that the relative order of persistence was simazine > atrazine > propazine(2-chloro-4,6-bis(isopropylamino)-s-triazine). Fink and Fletchall (27) working with several forage species observed that more severe injury was caused by atrazine than by simazine when the species were planted immediately after treatment. However, in plots seeded one year after application, simazine caused greater injury.

A number of researchers have reported differences in persistence of s-triazine herbicides among different soils (2,15,75,76). Scudder (76) found greater residual toxicity in a sandy soil than in a peat soil. This finding was confirmed by other workers (3) who found greater simazine residues in sandy soil than in a peat or clay soil up to 11

months after application.

Persistence of the chloro-s-triazines is modified by weather and climate through their effects on degradation. Both microbial and non-microbial decomposition is dependent on moisture and temperature. Warm, moist conditions have been shown to reduce persistence of s-triazines in soils (26,43), while in cold, dry climates these herbicides disappear more slowly (25,27,31). Burschel (16) showed that the half-life of simazine in soil at 8.5°C was 140 days, while at 25°C it was only 20 days.

Weather conditions within an area modify persistence patterns. Simazine and atrazine persist longer during dry summers than during summers with high rainfall (13,44,94). Rainfall and irrigation patterns also alter s-chloro-triazine persistence in soils. Working with three irrigation schemes, Wilson and Cole (106) demonstrated least atrazine persistence in soil that was watered daily to field capacity and greatest persistence in soil watered to field capacity once each week.

Formulation of the chloro-s-triazine herbicides appears to be related to persistence. Reports show that granular atrazine persisted longer than wettable powder formulations applied in aqueous suspension (59,107). Kuratle and Cole (54) also demonstrated that when atrazine was formulated as a water-soluble ammonium sulfate granule, it was considerably

more persistent than when it was formulated on insoluble Attaclay.

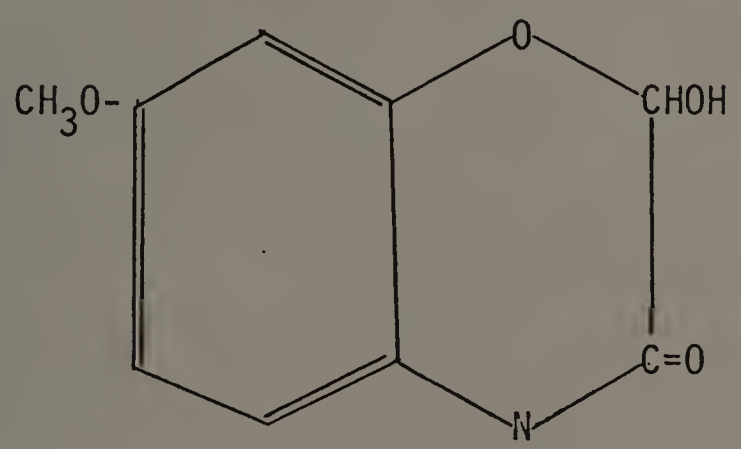
Atrazine is often applied in oil-water emulsion carriers when applied post-emergence (56,95,108). The effects of such carrier emulsions on persistence has not been fully investigated but Slife (85) suggests that residual problems may be greater when atrazine is applied with water as the carrier than when applied in an oil-water mixture.

Triazine Metabolism

A number of investigators have demonstrated a close correlation between the degree of plant resistance to the triazines and the extent of metabolism of these compounds (21,22,39,72,78). Roth was the first to observe that simazine was being extensively degraded by corn (72). He incubated simazine with expressed corn sap and noted that after 100 hours only a small percentage of the parent simazine could be detected in the mixture. On the other hand, over 90 percent of the simazine added to wheat sap was recovered in a similar experiment. This observation was highly significant inasmuch as wheat is sensitive to simazine. Montgomery and Freed (61) reported a rapid conversion of atrazine to a new compound, which on the basis of chromatographic behavior was suggested to be the hydroxy analog of atrazine. Castelfranco *et al*. (17) confirmed this, and

characterized the active constituent responsible for the conversion. The properties of this constituent suggest that it was not an enzyme or protein.

The constituent was later isolated and identified independently by two groups of researchers (42,73). It was found to be the cyclic hydroxamate, 2,4-dihydroxy -3-keto-7-methoxy-1, 4-benzoxazine.



2,4-dihydroxy-3-keto-7-methoxy-1,4-benzoxazine

Roth and Knusli (73) showed that simazine is detoxified *in vitro* in the presence of the cyclic hydroxamate, its glucoside and related cyclic hydroxamic acids. The *in vivo* (42) and *in vitro* (101) conversion of simazine to its hydroxy analog was also demonstrated. This conversion was thought to be common to all the chloro triazines, since propazine undergoes the same conversion when incubated in corn juice. The primary factor in the high tolerance of

corn to the s-triazine herbicides was at this time thought to be their rapid conversion to the hydroxy analogs. The hydroxy s-triazines are biologically inactive, even to species which are very susceptible to the chloro-s-triazines (30).

Metabolic studies have shown that susceptible plants have a limited capacity for degrading the chloro-triazines. Davis et al. (21), using ^{14}C-labeled simazine, showed that the amount of herbicide taken up by corn, cotton and cucumber plants did not vary significantly. However, after chloroform extraction (which removes unaltered simazine as well as certain metabolites) they discovered that only 5 percent of the radioactivity was chloroform-soluble where-as in cotton and cucumber plants the values were 25 percent and 50 percent respectively. The extent of simazine metabolism was in close agreement with the relative tolerances of these plants to the herbicide. Corn is very tolerant to simazine, cotton is moderately sensitive and cucumber is extremely susceptible.

The ability of corn plants to rapidly degrade simazine and atrazine was also demonstrated by Montgomery et al. (64). They grew plants in soil treated with ^{14}C herbicide and reported that the amount of chloroform-soluble as well as total radioactivity at each harvest (ranging from 2 weeks

to 3 months) was appreciable. However, the radioactivity in the chloroform-soluble fraction declined at each time of harvest, indicating that the triazines were metabolized. Analysis of the chloroform extracts by paper chromatography showed that very little, if any radioactivity, was still in the form of the original parent triazines.

The metabolism of ring and side-chain labeled simazine by corn plants was studied by Funderburk and Davis (33). Paper chromatography of the extract of treated plants revealed that hydroxy simazine and an unidentified ^{14}C product are formed with either type of labeled herbicide. Also labeled carbon dioxide was given off by plants treated with either compound. This indicated that all portions of the triazine molecule are subject to complete oxidation by corn, cotton and soybean plants.

Some doubt was raised however as to the relationship between degradation and tolerance in several species (40). Hamilton contended that although benzoxazinone derivatives may play a part in the resistance of corn and Jobs-Tears [Coix lacryma-jobi (L.)] to 2-chloro-4,6-dialkylamino-s-triazine herbicides, other factors appeared to be important. Kafer-60 Sorghum, which shows considerable resistance, does not contain benzoxazinone in detectable amounts, and did not appear to convert simazine to hydroxysimazine.

On the other hand, other susceptible species such as wheat and rye, contain benzoxazinone, and were capable of effecting the conversion (40). Pea plants, which show intermediate tolerance to atrazine was also found to be devoid of benzoxazinone (92). The presence of water-soluble metabolites other than hydroxy-simazine in the root extracts of barley, oats and sorghum indicated the possibility of other degradation mechanisms (40).

Contrary to the postulation that the first step in the degradation of atrazine involved hydroxylation at the 2-position (17,42,72), it was later shown that the moderately susceptible pea plant though incapable of converting atrazine to hydroxyatrazine, possessed an active system capable of converting the parent atrazine molecule into the dealkylated product, 2-chloro-4-amino-6-isopropylamino-s-triazine (compound 1). The results indicated that in the mature pea plant the principal degradation reaction of atrazine was the dealkylation of the ethyl group at the 4-position of the triazine molecule. The other possible matabolite, 2-chloro-4 amino-6-ethylamino-s-triazine, was not evident.

Shimabukuro (86) showed that the shoots of mature pea plants had a higher capacity to degrade atrazine than did the roots and that compound 1 was definitely less phytotoxic than atrazine to pea plants. The intermediate tole-

rance of pea plants to atrazine was therefore apparently due to their ability to convert the highly toxic atrazine rapidly to a less toxic metabolite, compound 1. The author suggested that in pea plants the accumulation or failure to metabolize atrazine to a completely non-toxic compound may keep the plants from being completely resistant to atrazine.

In a subsequent investigation it was found that dealkylation occurred not only in pea plants, but also in corn, wheat and sorghum (87). Both qualitative and quantitative differences in dealkylation of atrazine between the species were observed. All the species studied were able to metabolize atrazine initially by N-dealkylation of either of the 2-substituted alkyl-amine groups. N-dealkylation at the ethyl-amine side chain produced the isopropylamino derivative (compound 1), and N-dealkylation at the isopropylamine side chain resulted in the ethyl derivative (compound 2). Sorghum appeared to readily dealkylate either N-alkyl side chain, peas and wheat predominantly dealkylated the ethyl alkyl side chain.

Later work (89) confirmed the earlier suggestion that sorghum degraded atrazine via the N-dealkylation route, while in corn both the hydroxylation and N-dealkylation pathways existed. This researcher further showed that in corn, atrazine degradation led to three hydroxylated metabolites,

2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine (hydroxyatrazine), 2-hydroxy-4-amino-6-isopropylamino-s-triazine (hydroxycompound 1) and 2-hydroxy-4-amino-6-ethylamino-s-triazine (hydroxycompound 2). Hydroxycompounds 1 and 2 were formed in two ways in corn (1) by N-dealkylation of hydroxyatrazine and (2) by benzoxazinone- catalized hydrolysis of compound 1 and compound 2. Like hydroxyatrazine, hydroxycompounds 1 and 2 are non-phytotoxic.

Up to this point, the non-enzymatic hydrolysis of atrazine to hydroxyatrazine was accepted as the major factor responsible for the tolerance of corn to atrazine (17,39,42, 72). However, some researchers questioned this on the basis that no direct correlation existed between benzoxazinone concentration and tolerance to atrazine and simazine in selected corn lines (41,66).

Shimabukuro and Swanson (93) working with sorghum, demonstrated a rapid conversion of atrazine to a water-soluble compound (metabolite B) resulting in a rapid recovery of photosynthetic activity. The subsequent isolation and identification of the water-soluble metabolite B as a mixture of S-(4-ethylamino-6-isopropylamino-2-s-triazino) glutathione and γ-glutamyl-s-(4-ethylamino-isopropylamino-2-s-triazino) cysteine, revealed the presence of a third detoxification pathway in higher plants (55). The discovery of this path-

way as an active detoxification mechanism found in highly tolerant corn and sorghum (55,91,93) appears to solve the inconsistencies observed between benzoxazinone content and tolerance to atrazine.

Further investigation (90) established that the main factor responsible for tolerance in corn was the activity of a soluble enzyme, glutathione-S-transferase. They demonstrated the accumulation of significant amounts of unchanged atrazine in the susceptible corn line, GT112 which showed a low rate of glutathione conjugation because of low glutathione-S-transferase activity. An assay of glutathione-S-transferase in leaf tissue and results of atrazine degradation indicated that most corn plants may be totally tolerant to atrazine even if the tissues are devoid of benzoxazinone. They also noted that the enzymatic glutathione conjugation reaction occurs primarily in the shoots, while the non-enzymatic hydroxylation reaction occurs primarily in the roots of corn plants. Significant amounts of hydroxyatrazine was formed only when atrazine was initially absorbed through the root where the enzymatic reaction does not compete for the atrazine substrate. Frear and Swanson (29) had earlier detected little or no glutathione-S-transferase activity in corn root tissue. Glutathione conjugation is recognised as the initial

reaction leading to the biosynthesis of mercapturic acid, and this pathway is known to be a means for detoxication and excretion of foreign compounds in animals (12).

Materials and Methods

Uptake, translocation and metabolism of s-triazines by witchgrass and crabgrass.

Plant Material

Witchgrass and crabgrass seeds were germinated on blotting paper (moistened in 0.2 percent KNO_3 to break seed dormancy) in an alternating dark (15°C)/light (30°C) environment. The seedlings were then transplanted and grown to the 2nd and 4th leaf stages in sand - nutrient solution culture in a growth chamber at 30°C day and 27°C night temperatures and a photoperiod of 14 hours.

Incubation and Extraction

On reaching the desired stages the plant roots were carefully washed free of sand and the plants incubated in 250 ml. of 1.1×10^{-6} M solutions of uniformly ring-labeled ^{14}C-simazine and ^{14}C-atrazine (specific activity 10.1μCi/mg and 12.5μCi/mg respectively) made up in one-half strength Hoagland's solution. Plants were incubated for periods of 2 to 24 hours (continuous light) in a controlled environment room maintained at 28 ± 2°C and a 50 ± 5 percent relative humidity. The light intensity was 650 foot candles at the level of the shoot apex during the

treatment period. Each treatment was replicated ten times. There were five plants in each replication.

At the end of the treatment period, the plants were removed from the ^{14}C-s-triazine-nutrient solution, rinsed briefly in running water, separated into shoots and roots and weighed. The plant parts were homogenized in 100 ml. of 90 percent methanol and filtered. The residue was re-extracted twice by resuspending in 90 percent methanol. Methanol was removed under vacuum at 30°C and the remaining aqueous solution centrifuged at 13,300 G for 15 minutes at 0°C to remove plant debris. The aqueous supernatant was concentrated to 10 ml. A 0.5 ml. aliquot was removed, added to 20 ml. of scintillation liquid (5g/l PPO, 100g/l naphthalene, dioxane - balance of liter) and assayed for ^{14}C activity by liquid scintillation counting, using a Beckman Model LS-100 system. All samples were corrected for quenching and the amount of s-triazine was determined from the total activity present and the specific activity of the ^{14}C-s-triazine.

The remaining aqueous extract was partitioned four times between water and chloroform to remove unchanged s-triazine and chloroform-soluble metabolites. Aliquots of the aqueous and chloroform fractions were assayed for ^{14}C

activity by liquid scintillation counting as previously described.

Qualitative Determinations

The aqueous ^{14}C fraction was further purified by retention on a Dowex 50W - X8 (H^+), water-jacketed column (40 ml. capacity) and the column was washed with water (92). The radioactivity was eluted with 30 ml. of 10N NH_4OH. Ammonia was removed under vacuum at 30°C and the purified water-soluble fraction was spotted together with reference compounds on silica gel HF thin-layer plates (50μ) and the plates developed in solvent A (isopropanol:30% ammonium hydroxide:water (8:1:1)) and solvent B (N-butanol:acetic acid:water (12:3:5)). The chloroform fraction was also spotted on 50μ silica gel thin-layer plates and developed in solvents A and B.

Preparation of Metabolite Standards

Radioactive hydroxyatrazine and hydroxysimazine used as chromatographic reference compounds were prepared by hydrolysis of atrazine and simazine in equal amounts of 6N HCl and 95 percent ethanol at 50°C for eight hours (40).

Radioactive peptide conjugates used in the study were prepared after the method of Shimabukuro et al. (88,91).

The fourth leaf (30 - 40 cm.) of 24-day-old corn plants was excised under water. The leaves were treated for 24 hours by immersing the cut ends into test tubes each containing 4 ml. of ^{14}C-atrazine or ^{14}C-simazine solution (approxmately 400,000 dpm). The level in the test tubes were maintained by adding distilled water intermittently to compensate for water loss due to uptake and transpiration. The metabolites were extracted and purified by thin-layer chromatography as previously described. The chromatogram was developed in n-butanol:acetic acid:water (12:3:5) and the radioactivity in the region corresponding to the peptide conjugates were scraped off and purified as earlier described.

Relative Toxicity Studies

a. Growing Media - Soil

Witchgrass and crabgrass seeds were germinated in petri dishes on KNO_3 - moistened blotting paper as previously described. The seedlings were transplanted into 4 inch plastic pots containing 500g (on an oven dried basis) of Woodbridge soil (58.8%sand, 8.8% clay, 3.7% organic matter). The plants were grown in a growth chamber under the conditions previously described, and treated with 100 ml. of 0 - 8 ppm solutions of simazine and atrazine (made up in one-

half strength Hoagland's solution) applied as a root drench at the 2-leaf and 4-leaf stages. A randomized block design was used with five replications per treatment and five plants per replication. Each pot was watered with 50 ml. of one-half strength Hoagland's solution every other day.

Above ground parts of the plants were harvested after 14 days of treatment and the dry weights determined. The results were expressed as percent of the control and plotted on graph paper as a function of the concentration. The ED_{50}(D.W.) values (the concentration of herbicide which reduced dry weight 50 percent) were estimated (97).

b. Growing Media - Sand

A procedure similar to that outlined above was followed, except seedlings were transplanted into pots containing 500g. of washed river sand. Plants were treated at the same growth stages with 50 ml. of 0 - 16 ppm of simazine and atrazine made up in one-half strength Hoagland's solution. Growing conditions during the experiment and dry weight determinations were as reported for the soil grown study.

Assay of Glutathione-S-Transferase

a. Enzyme Preparation

5g excised leaf tissue was rinsed with water, blotted dry and pulverized in liquid N_2. The frozen powder was

slurred with 2.5g of Polyclar A T (insoluble polyvinylpyrolidione) in 25 ml. of 0.1 M phosphate buffer, pH 6.8. Sodium metabisulfite was added to the buffer to give a 1.0 mM final concentration. The slurry was allowed to stand for 15 minutes, squeezed through four layers of cheese cloth and centrifuged at 17,500G for 30 minutes. The supernatant was then fractionated between 30-60 percent saturation with ammonium sulfate.

The precipitate was suspended in 1.0 ml. of 0.1M phosphate buffer, pH 6.8, dialysed against the same buffer for two hours, lyophilized in test tubes, and stored dry at -12°C.

b. Enzyme Assay

The enzyme assay was based on the rate of glutathione conjugate formation. The standard reaction mixture was made up as follows:

0.1 ml. of enzyme (0.68-0.83mg of protein).

30.5 n moles of ^{14}C-atrazine.

0.1 ml. of 1.0 M phosphate buffer, pH 6.8.

0.1 ml. of glutathione (10μmoles) reduced form.

H_2O to give a final volume of 1.0 ml.

The reaction was initiated by the addition of substrates and incubated at 25° for 30 minutes. Controls were heated in a boiling water bath for 15 minutes and cooled to room

temperature prior to the addition of substrates. The reaction was terminated by rapid freezing in a dry-ice-acetone bath followed by lyophilization.

The lyophilized reaction mixture was extracted with 0.6 ml. of MeOH and the ^{14}C-labeled glutathione conjugate was separated from the remaining ^{14}C-labeled substrate by spotting a 150µl aliquot of the MeOH extract on SiO_2 HF plates (50µ). The plates were developed to a height of 15 cm with two ascending solvent systems (1) benzene:acetic acid:water (60:40:3) and (2) n-butanol:acetic acid:water (12:3:5).

Radioactive 2-hydroxy-4-ethylamino-6-isopropylamino-s-triazine (10µg) was also spotted on each plate as a reference compound. The area between the reference marker and the origin was scraped off the chromatogram (Rf 0 - 0.64), and the ^{14}C-labeled glutathione conjugate quantitatively determined by liquid scintillation counting.

The enzyme unit (U) was defined as the amount of enzyme required for the biosynthesis of 1 Mu mole of glutathione conjugate per 30 minutes under standard assay conditicns.

Protein was determined by the method of Lowry et al. (58) with crystalline bovine serum albumin used as the standard.

RESULTS

Susceptibility Studies

Dosage response curves of soil-grown crabgrass and witchgrass to simazine and atrazine (figures 1 and 2) show that both species are less susceptible with advanced development. The ED_{50} value for atrazine to 4-leaf crabgrass is 8.0 ppm while to the 2-leaf stage, the value is 6.9 ppm. Witchgrass followed a similar trend with ED_{50} values of 4.4 ppm and 3.8 ppm for the 4-leaf and 2-leaf stages respectively.

Crabgrass at both stages of development is more resistant to the s-triazine herbicides than is witchgrass, since the ED_{50} for both stages is always reached at a lower concentration. Simazine is more effective than atrazine in reducing dry weight (figures 1 and 2). The ED_{50} value for 4-leaf crabgrass to atrazine is 8.0 ppm, while that for simazine is only 1.5 ppm. This pattern is similar for the 2-leaf crabgrass stage as well as for the two witchgrass stages.

The results of toxicity studies for sand-grown plants (figures 3 and 4) are in relative agreement with those for the soil-grown plants. (1) Both species show decreased susceptibility with development. (2) Crabgrass is more

Figure 1. Dosage response curves of soil-grown crabgrass and witchgrass to atrazine.

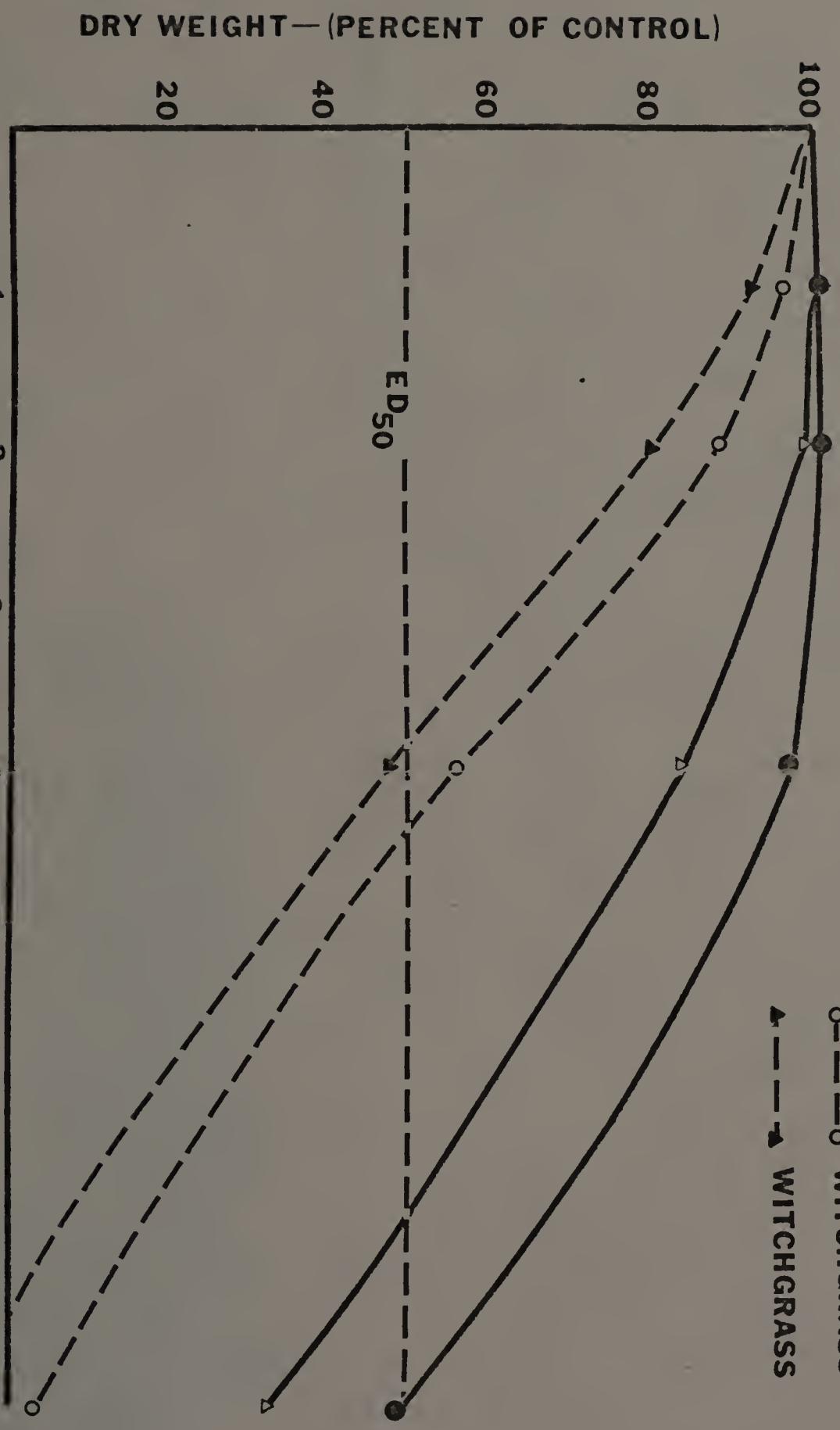
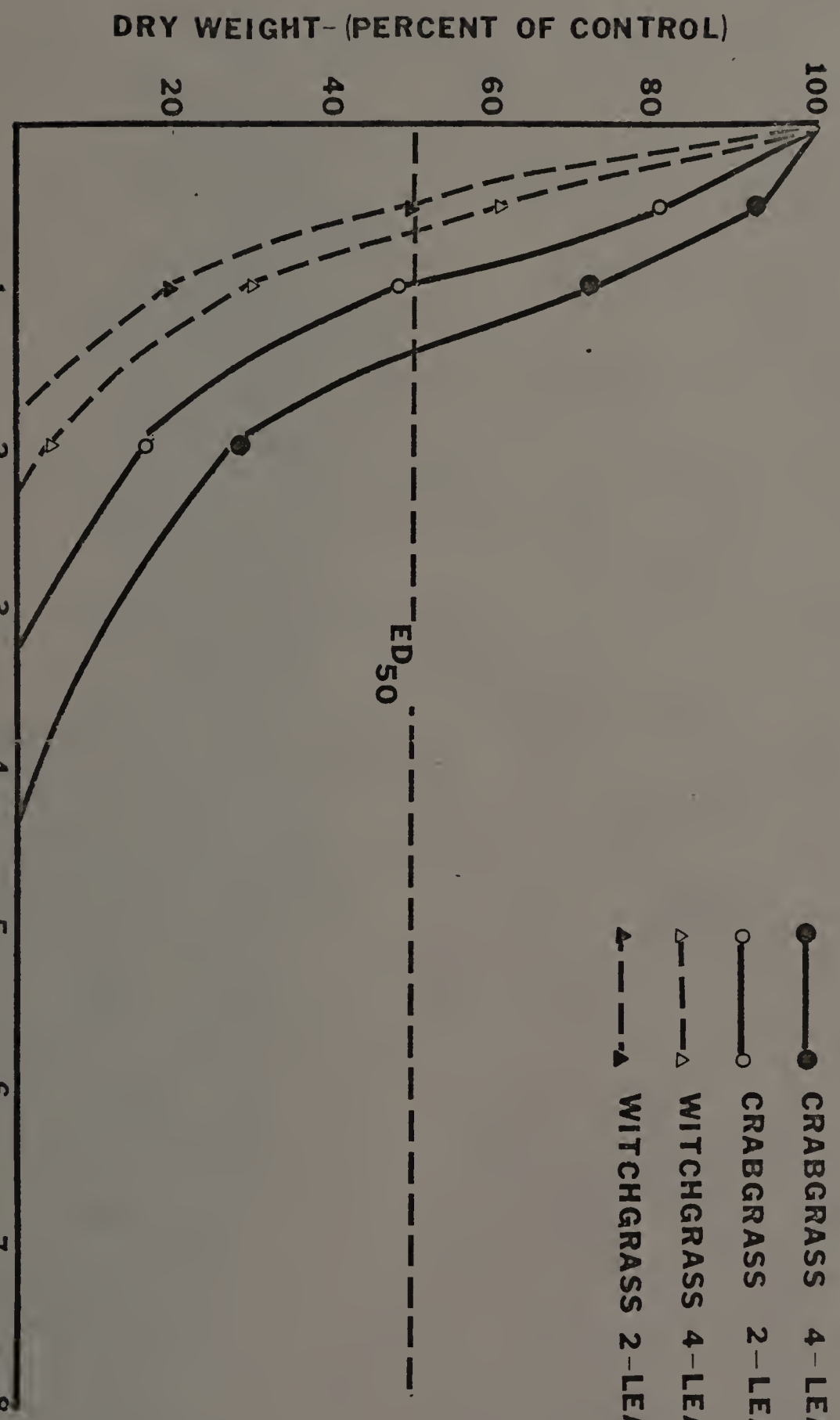


Figure 2. Dosage response curves of soil-grown crabgrass and witchgrass to simazine.



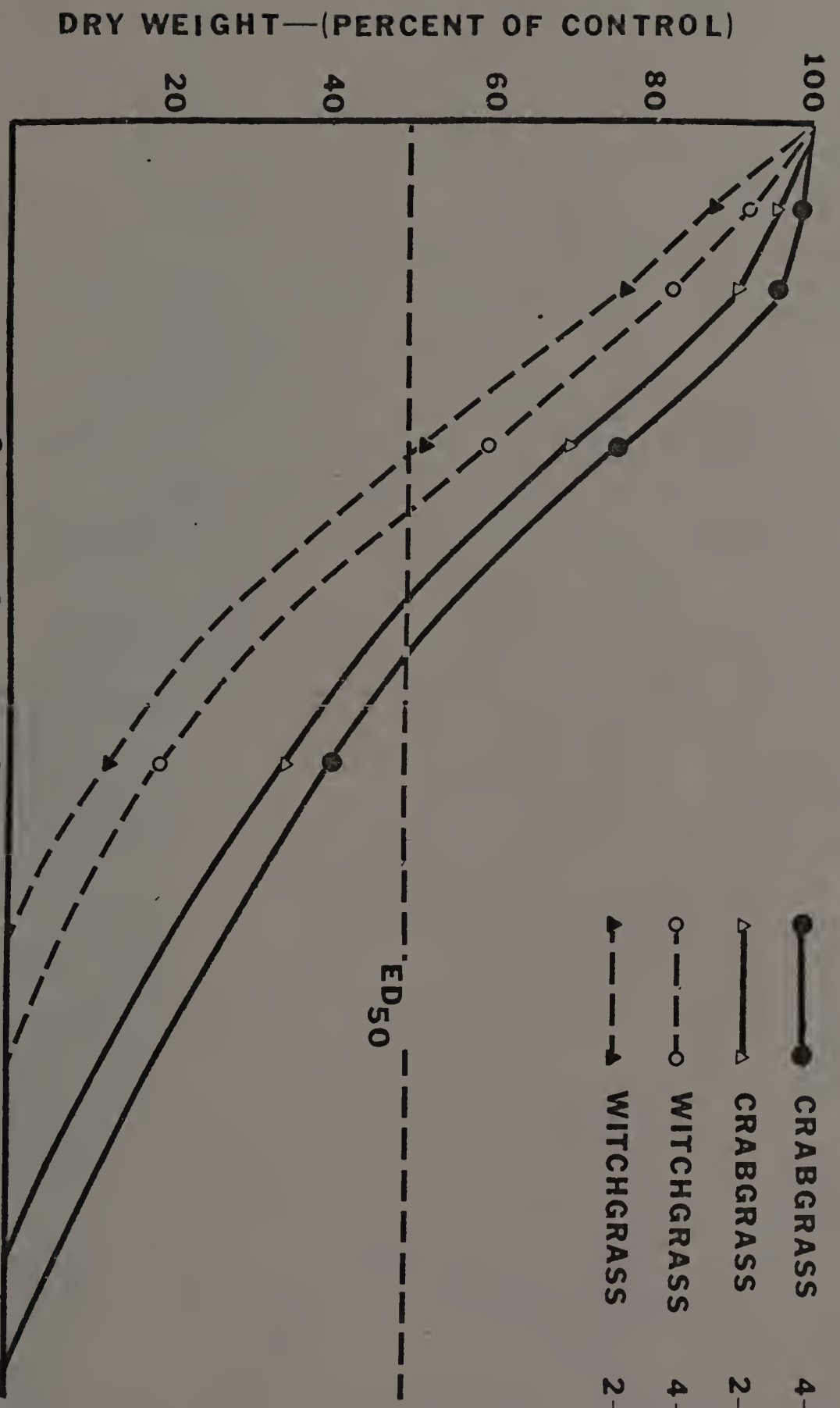
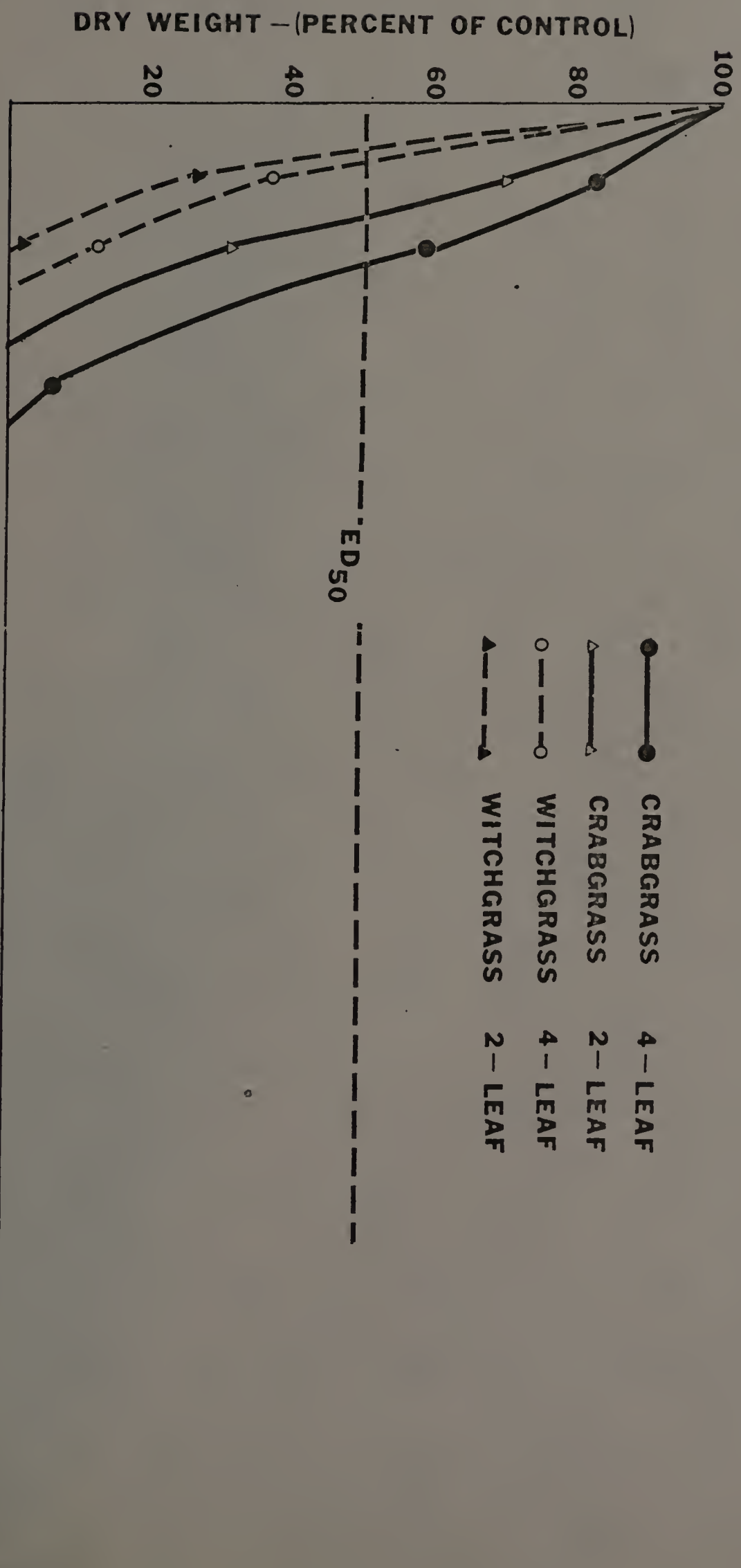


Figure 3. Dosage response curves of sand-grown crabgrass and witchgrass to atrazine.

Figure 4. Dosage response curves of sand-grown crabgrass and witchgrass to simazine.



tolerant than witchgrass to the s-triazine herbicides.
(3) Simazine shows greater toxicity than atrazine to both species. However, as expected, the ED_{50} values for sand-grown plants of both species are lower than those for soil-grown plants.

Uptake Studies

Table 1 shows the uptake of ^{14}C-simazine and ^{14}C-atrazine over a 24 hour incubation period. The data shows that simazine and atrazine are absorbed in similar quantities by both plants. Also, the amounts of ^{14}C activity found in the two plant species is not appreciably different.

The pattern of uptake of simazine and atrazine by crabgrass and witchgrass are also similar (figure 5), the uptake of the s-triazine herbicides after 2,4,12 and 24 hours not differing signigicantly.

Metabolism of s-triazines

Both witchgrass and crabgrass metabolized simazine and atrazine to water-soluble derivatives (Table 2). However, neither plant appeared to convert simazine extensively to hydrophilic metabolite(s). Approximately 40 percent of the total ^{14}C-atrazine absorbed by crabgrass in 24 hr was converted to hydrophilic metabolite(s), where-

Table 1. Uptake of ^{14}C-simazine and ^{14}C-atrazine by crabgrass and witchgrass over a 24 hour period from $1.1 \times 10^{-6}M$ solutions.

	Moles^{-10} per g of fresh weight	
Herbicide	Crabgrass	Witchgrass
Simazine	125.5a	129.8a
Atrazine	127.1a	124.1a

Means within a column followed by a different letter are significantly different at the 5% level.

Figure 5. Time course uptake of ^{14}C-atrazine and ^{14}C-simazine by crabgrass and witchgrass from 1.6×10^{-6}M solutions.

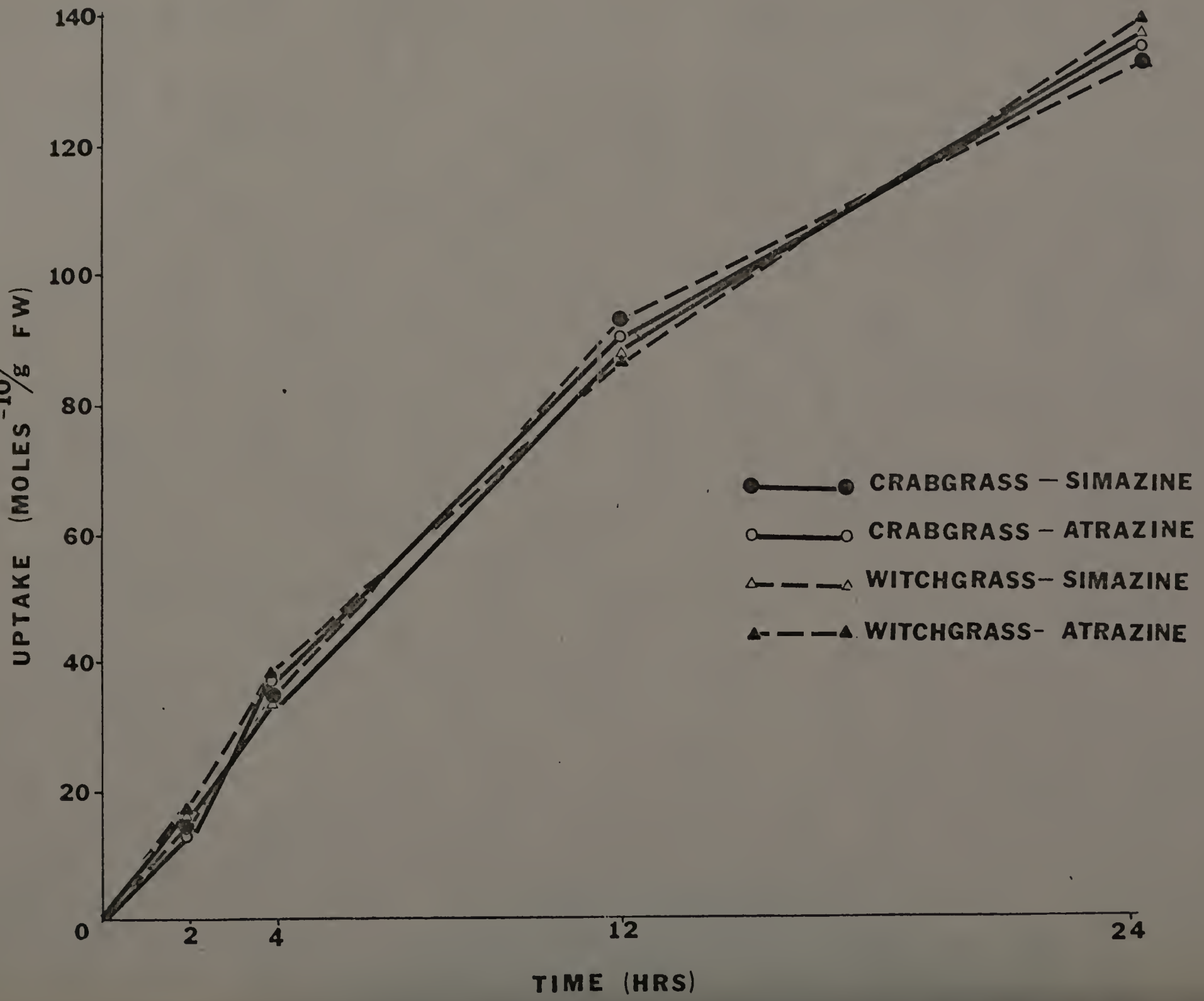


Table 2. Metabolism of ^{14}C-simazine and ^{14}C-atrazine during a 24 hour period from 1.1×10^{-6}M solutions.

	Simazine		Atrazine	
	Moles^{-10} per g fresh weight			
Fraction	Witchgrass	Crabgrass	Witchgrass	Crabgrass
Hydrophilic	5.6a	8.6a	29.7b	50.8c
Chloroform-soluble	124.2a	116.8a	94.1b	76.7c

Means in a row followed by a different letter are significantly different at the 5% level.

as the value for witchgrass was about 24 percent. It is apparent that (1) the two plant species were less able to degrade simazine than atrazine and (2) crabgrass converted far greater amounts of atrazine to hydrophilic metabolite(s) than did witchgrass.

Figures 6 and 7 show the time course uptake and metabolism of ^{14}C-simazine and ^{14}C-atrazine by crabgrass and witchgrass. No differences in the s-triazine concentration in atrazine and simazine-treated plants were found after 2,4,12 or 24 hour incubation periods. No hydrophilic metabolite(s) of simazine could be detected after a 4 hr incubation period in witchgrass, while after the same period only trace amounts were detected in crabgrass. At no time period were there significant differences in the quantities of hydrophilic metabolite(s) of simazine in the two plant species.

Atrazine metabolism on the other hand was apparent in both plant species as early as the 2 hr period. Atrazine metabolism occurred at a faster rate in crabgrass plants than in witchgrass, and there were significantly more hydrophilic metabolite(s) in crabgrass than in witchgrass after 4,12 and 24 hour incubation periods.

Figure 6. Time course uptake and metabolism of ^{14}C-simazine by crabgrass and witchgrass from 1.6 x 10^{-6}M solutions.

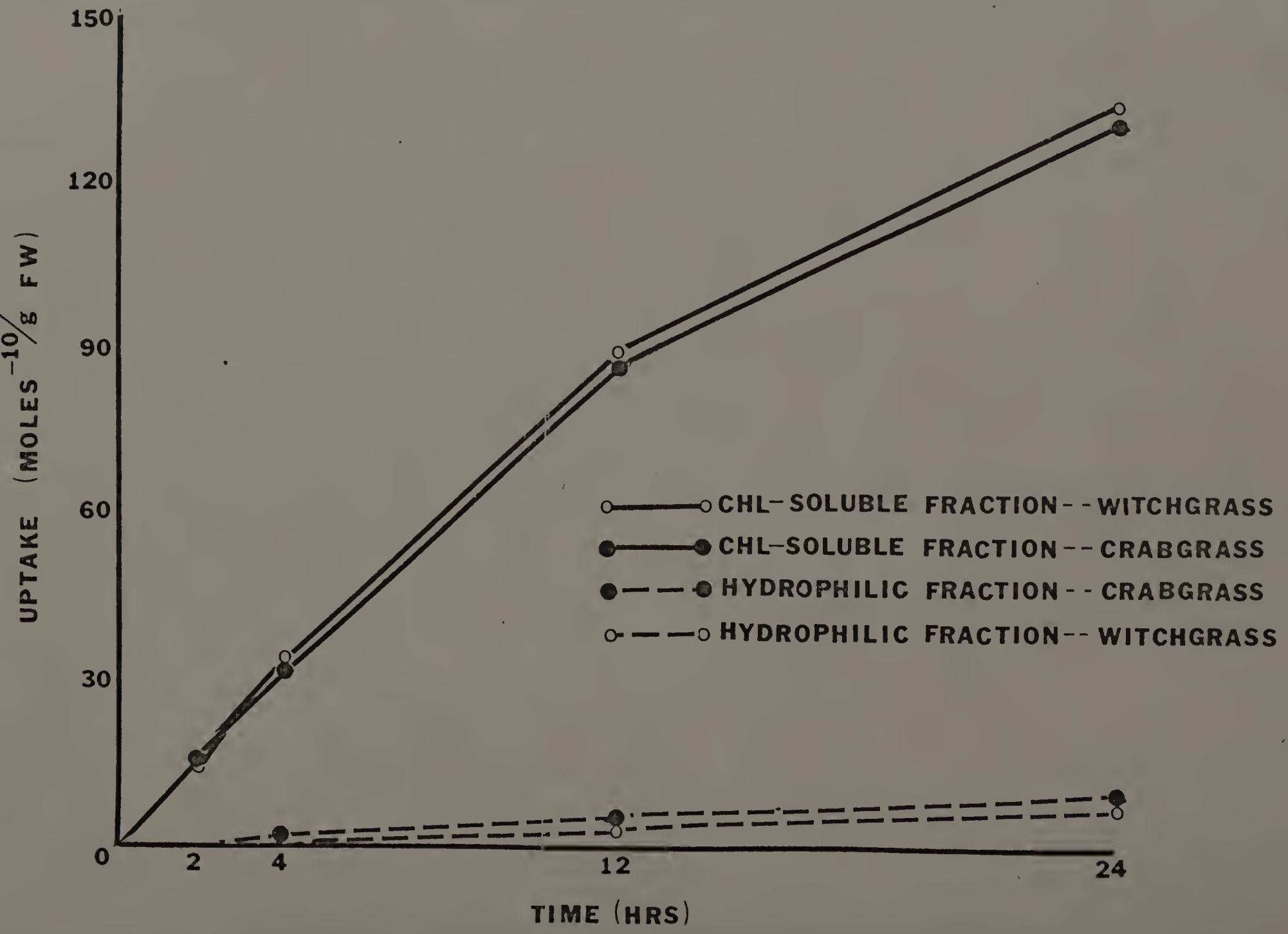
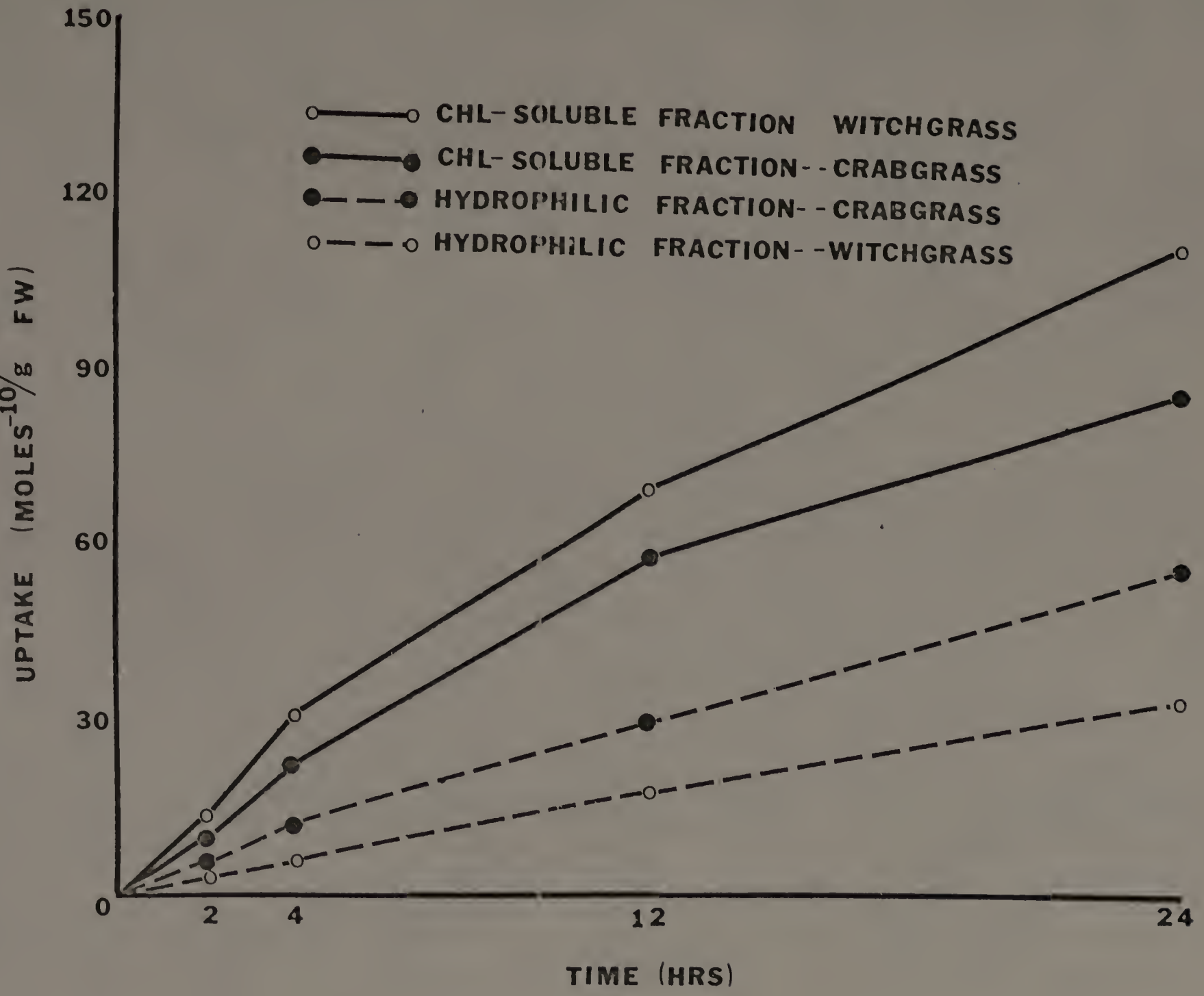


Figure 7. Time course uptake and metabolism of ^{14}C-atrazine by crabgrass and witchgrass from 1.6×10^{-6} M solutions.



Translocation

Simazine and atrazine are translocated similarly in crabgrass and witchgrass (Table 3). However, there was consistently larger quantities of ^{14}C activity in the shoots of witchgrass than in the shoots of crabgrass, and correspondingly, more ^{14}C activity in crabgrass roots than in the same tissue of witchgrass.

Identification of Metabolites

The Rf values of standards and metabolites are shown in Table 4. Practically all the radioactivity in the roots of both species chromatographed as unchanged s-triazine (Figures 9, 11, 13, 15). The chloroform-soluble fractions from both plants have Rfs corresponding to atrazine and simazine. The major hydrophilic metabolite from crabgrass and witchgrass is identical, with Rfs of 0.20-0.23 in solvent A and 0.41-0.44 in solvent B. Further investigation revealed that the major metabolite from atrazine treated plants (Figures 10, 14) are similar to the hydrophilic metabolite recently isolated from corn and sorghum (29,55,90,91) and thought to be peptide conjugates, S-(ethylamino-6-isopropylamino-2-s-triazino) glutathione, γ-glutamyl-S-4-ethylamino-6-isopropylamino-2-s-triazino) cysteine, or both. The behavior of the major hydrophilic metabolite from simazine

Table 3. Uptake and translocation of ^{14}C-simazine and ^{14}C-atrazine by crabgrass and witchgrass during a 24 hour incubation period from 1.1 x 10^{-6}M solutions.

Herbicide	Plant	Shoot	Root
		Moles $^{-10}$ of s-triazine per g of fresh weight	
Simazine	Crabgrass	75.3a	50.3a
	Witchgrass	94.9b	35.1b
Atrazine	Crabgrass	78.6a	48.7a
	Witchgrass	91.3b	32.9b

Means within a column followed by a different letter are significantly different at the 5% level.

Table 4. Rf values of standards and metabolites by ascending chromatography.

Substance	Solvent system A[1]	Solvent system B[2]
Atrazine	0.68 - 0.70	0.71 - 0.73
Simazine	0.67 - 0.68	0.68 - 0.70
Hydroxyatrazine	0.58 - 0.60	0.57 - 0.58
Hydroxysimazine	0.61 - 0.63	0.59 - 0.62
Peptide conjugate(s) of atrazine	0.21 - 0.23	0.41 - 0.43
Atrazine metabolite(s) from shoot of crabgrass	0.20 - 0.23	0.41 - 0.44
Atrazine metabolite(s) from shoot of witchgrass	0.21 - 0.22	0.41 - 0.43
Atrazine metabolite from root of crabgrass	0.69 - 0.70	0.71 - 0.72
Atrazine metabolite from root of witchgrass	0.68 - 0.71	0.71 - 0.73
Simazine metabolite(s) from shoot of crabgrass	0.21 - 0.22	0.41 - 0.43
Simazine metabolite(s) from shoot of witchgrass	0.20 - 0.23	0.40 - 0.42
Simazine metabolite from root of crabgrass	0.66 - 0.69	0.69 - 0.70
Simazine metabolite from root of witchgrass	0.67 - 0.69	0.68 - 0.70

[1]Isopropanol:30% ammonium hydroxide:water (3:1:1)

[2]N-butanol:acetic acid:water (12:3:5)

treated crabgrass and witchgrass plants during thin-layer chromatography was similar to the peptide conjugate(s) of atrazine from corn, crabgrass and witchgrass (Figures 8, 12).

Glutathione-s-transferase activity

The leaves of field corn (Table 5.) show considerably higher glutathione-s-transferase activity than those of the two other grass species studied. The activity of the enzyme is higher in crabgrass (0.63) than in witchgrass (0.38).

Standard errors did not exceed 5% and have been omitted from preceding figures.

Table 5. Glutathione S-transferase activity in corn crabgrass and witchgrass leaves.

Plant	Special Activity
	nmoles GS-atrazine per mg protein per hr/
Field Corn	3.10
Crabgrass	0.63
Witchgrass	0.38

Figure 8. Autoradiogram of thin-layer chromatograms of water-soluble metabolites of simazine-^{14}C in crabgrass and witchgrass after an initial 24 hour exposure period. Thin layer plate developed in isopropanol:30% ammonium hydroxide: water (8:1:1 v/v/v). No.1-hydrophilic metabolites extracted from corn plants treated with simazine, No.2-hydrophilic metabolites from witchgrass plants treated with simazine, No.3-hydrophilic metabolites from crabgrass plants treated with simazine,No. 4-authentic simazine, No. 5-authentic hydroxysimazine.
A-simazine. B-hydroxysimazine. C-unidentified simazine metabolite. D-GS-simazine. E-unidentified simazine metabolite. O-origin.

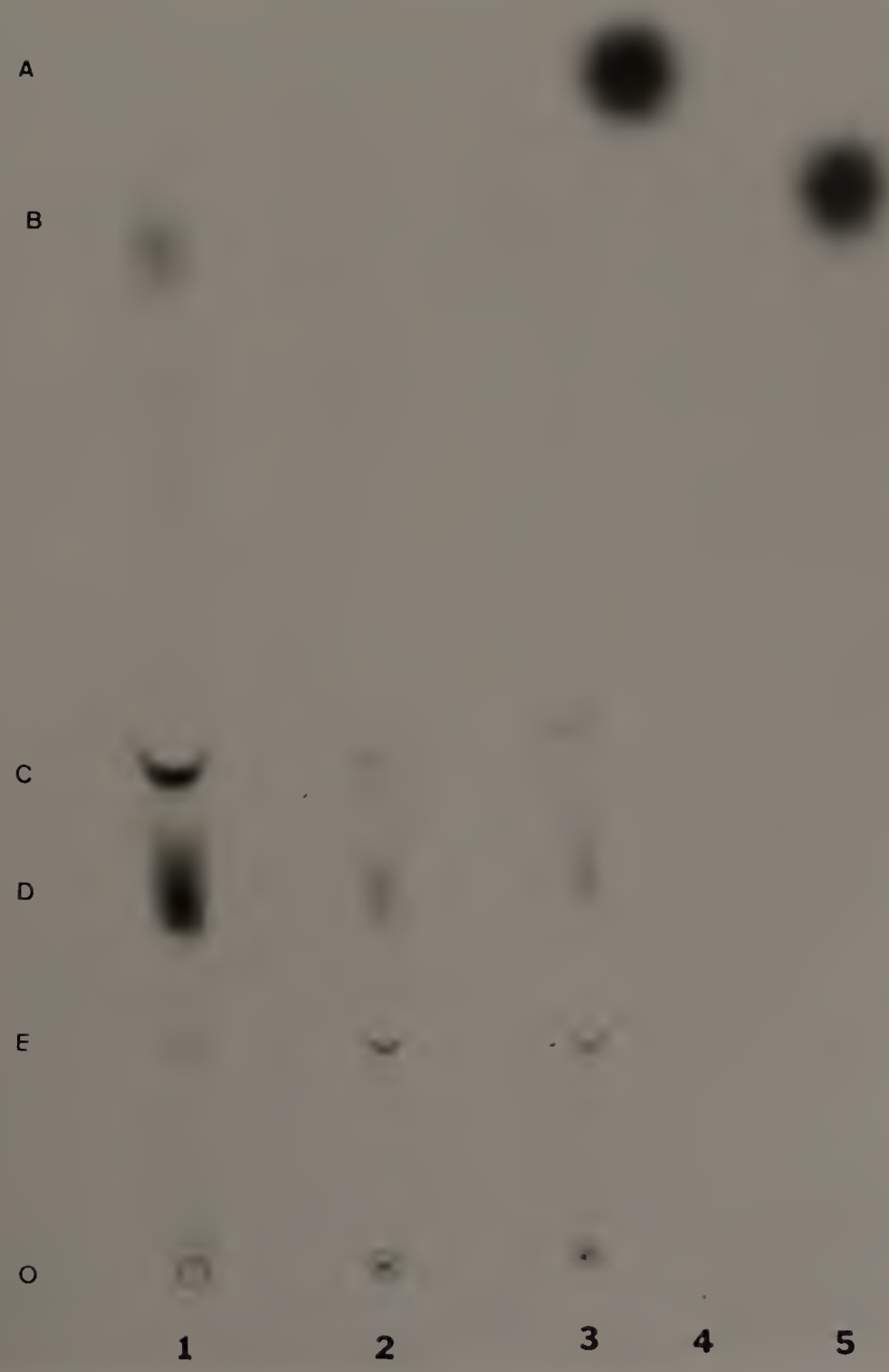

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Figure 9. Autoradiogram of thin-layer chromatogram of chloroform-soluble metabolites of simazine-^{14}C in crabgrass and witchgrass after an initial 24 hour exposure period. Thin layer plate developed in isopropanol: 30% ammonium hydroxide: water (8:1:1 v/v/v).
No. 1-authentic simazine, No.2-chloroform-soluble metabolite from witchgrass, No.3-chloroform-soluble metabolite from crabgrass. O-origin.

0

1 2 3

Figure 10. Autoradiogram of thin-layer chromatogram of water-soluble metabolites of atrazine-^{14}C in crabgrass and witchgrass plants after an initial 24 hour exposure period. Thin layer plate developed in isoproanol: 30% ammonium hydroxide: water (8:1:1 v/v/v). No.1-hydrophilic metabolites extracted from corn plants treated with atrazine, No.2-hydrophilic metabolites from witchgrass plants treated with atrazine, No.3-hydrophilic metabolites from crabgrass plants treated with atrazine, No.4-authentic atrazine, No.5-authentic hydroxyatrazine.
A-atrazine. B-hydroxyatrazine. C-unidentified atrazine metabolite. D-GS-atrazine. E-unidentified atrazine metabolite. O-origin.


A
B

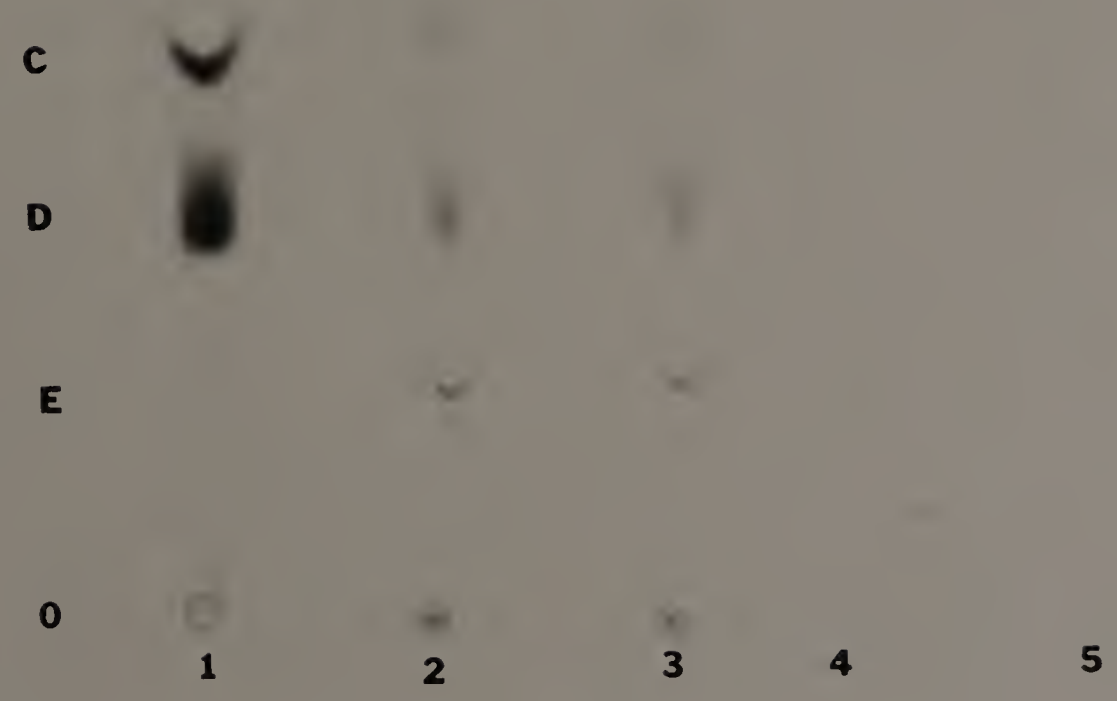
C
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Figure 11. Autoradiogram of thin-layer chromatogram of chloroform-soluble metabolites of atrazine-^{14}C in crabgrass and witchgrass after an initial 24 hour exposure period. Thin layer plate developed in isopropanol:30% ammonium hydroxide: water (8:1:1 v/v/v). A-authentic atrazine. B-chloroform soluble metabolite from witchgrass. C-chloroform soluble metabolite from crabgrass. O-origin.

0 1 2 3

Figure 12: Autoradiogram of thin-layer chromatogram of water-soluble metabolites of simazine-^{14}C in crabgrass and witchgrass after an initial 24 hour exposure period. Thin layer plate developed in n-butanol: acetic acid:water (12:3:5 v/v/v). No.1-hydrophilic metabolites extracted from corn plants treated with simazine No. 2-hydrophilic metabolites from witchgrass plants treated with simazine, No.3-hydrophilic metabolites from crabgrass plants treated with simazine, No.4-authentic simazine, No. 5-authentic hydroxysimazine.
A-simazine. B-hydroxysiamzine. C-GS-simazine
D-unidentified simazine metabolite. O-origin.

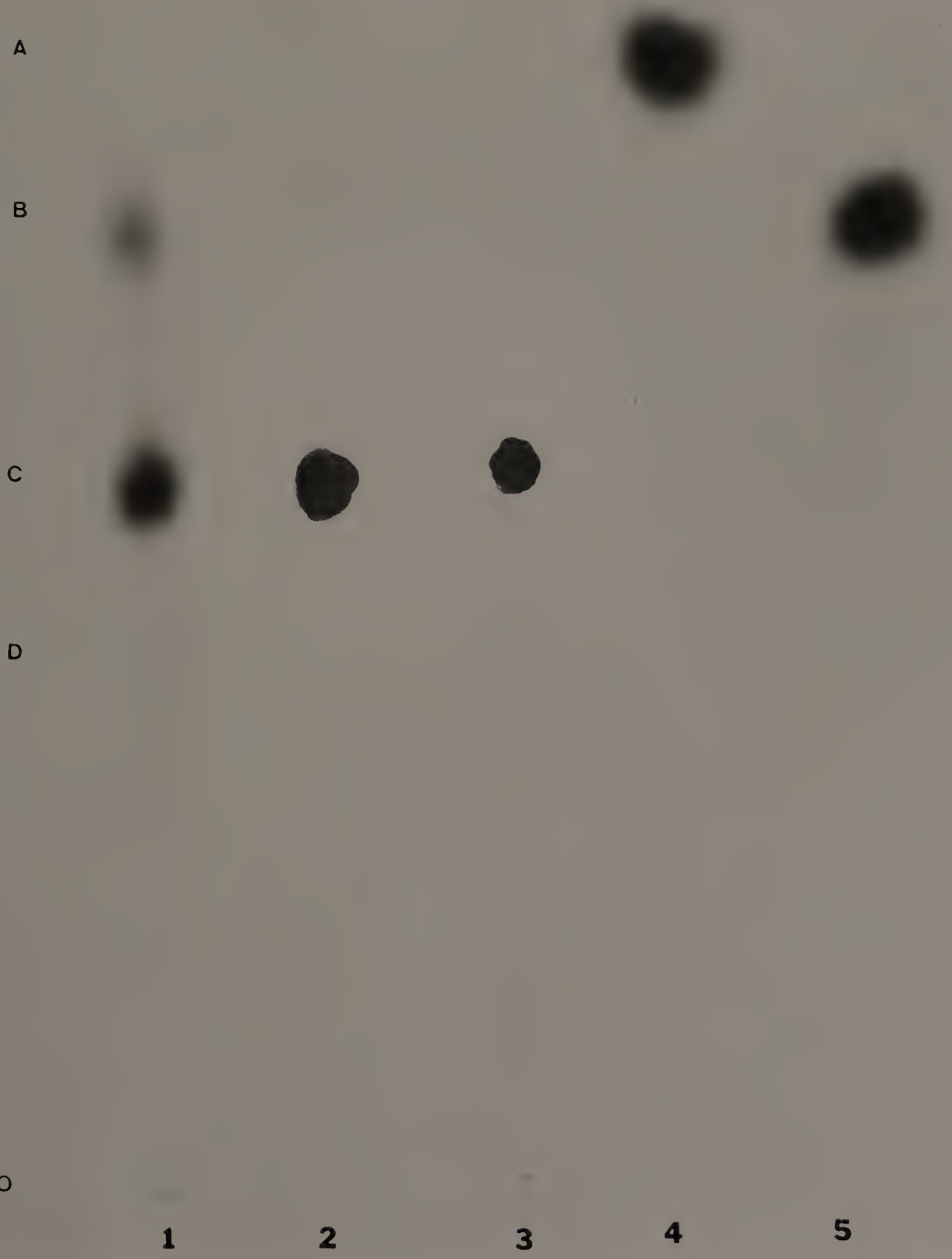
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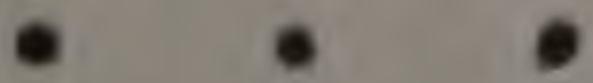
Figure 13. Autoradiogram of thin-layer chromatogram of chloroform-soluble metabolites of simazine in crabgrass and witchgrass after an initial 24 hour exposure period. Thin layer plate developed in n-butanol: acetic acid:water (12:3:5 v/v/v/). 1-authentic simazine. 2-chloroform-soluble metabolite from witchgrass. 3-chloroform-soluble metabolite from crabgrass. O-origin.



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1 2 3

Figure 14. Autoradiogram of thin-layer chromatogram of water-soluble metabolites of atrazine-^{14}C in crabgrass and witchgrass after an initial 24 hour exposure period. Thin layer plate developed in n-butanol: acetic acid:water (12:3:5 v/v/v). No.1-hydrophilic metabolites extracted from corn plants treated with atrazine, No.2-hydrophilic metabolites from witchgrass plants treated with simazine, No.3-hydrophilic metabolites from crabgrass plants treated with atrazine, No.4-authentic atrazine, No.5-authentic hydroxyatrazine.
A-atrazine. B-hydroxyatrazine. C-GS-atrazine D-unidentified atrazine metabolite. E-unidentified atrazine metabolite. O-origin.

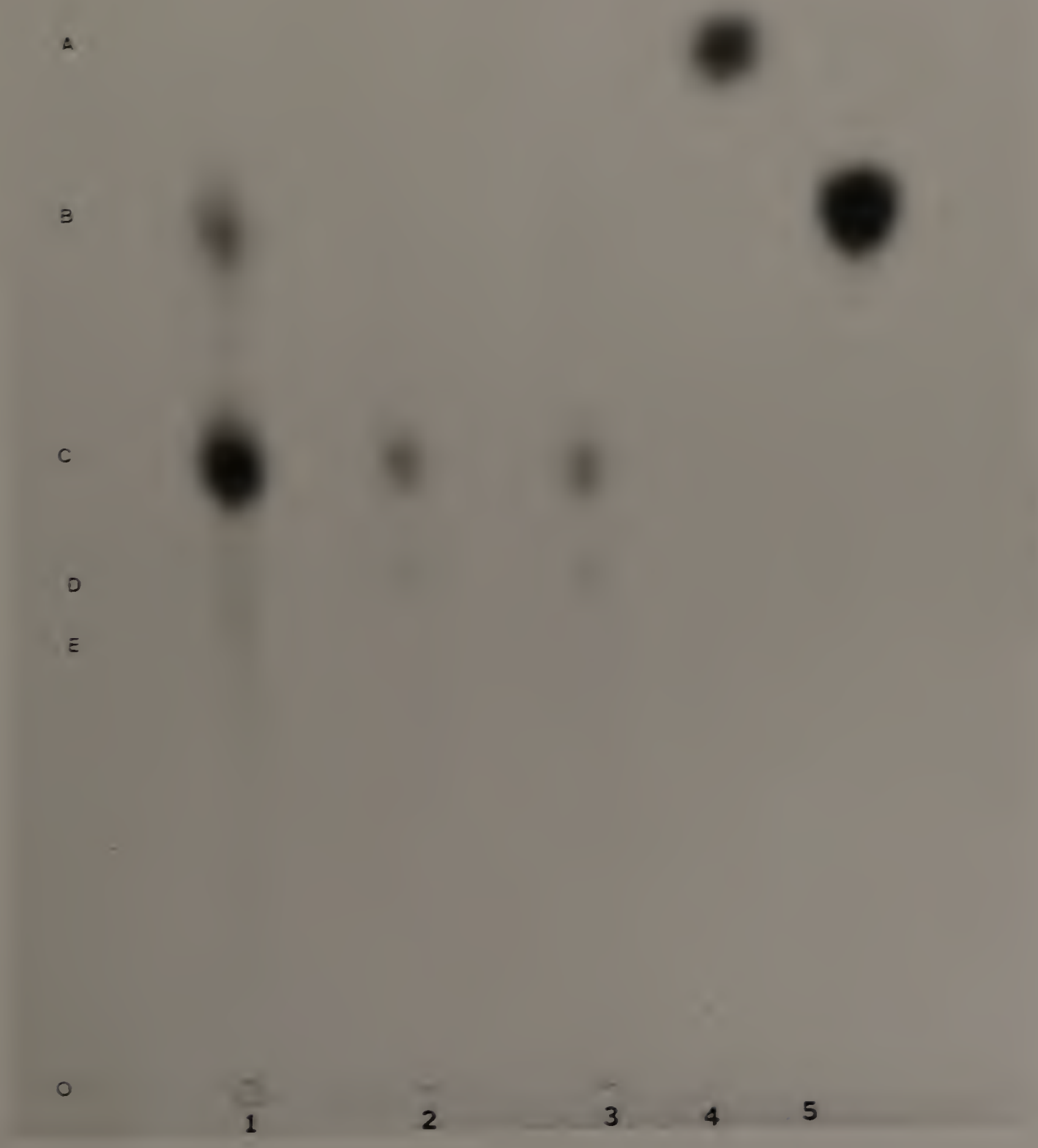
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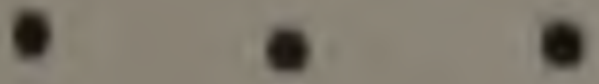
Figure 15. Autoradiogram of thin-layer chromatogram of chloroform-soluble metabolites of atrazine-^{14}C in crabgrass and witchgrass after an initial 24 hour exposure period. Thin layer plate developed in n-butanol: acetic acid: water (12:3:5 v/v/v).
1-authentic atrazine. 2-chloroform-soluble metabolite from witchgrass. 3-chloroform-soluble metabolite from crabgrass. O-origin.



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D I S C U S S I O N

The susceptibility studies clearly show crabgrass to be the more tolerant of the two grass species to the s-triazine herbicides. This is in agreement with field reports (67) in which atrazine was found to give significant control of witchgrass but essentially no control of crabgrass. The data also indicates that simazine is more toxic to both plants than is atrazine. This finding also supports earlier work which showed atrazine to be less toxic than simazine to wild cane in the field (100). Since however atrazine has been found to be more effective than simazine in inhibition of photosynthesis (37), it is evident that some other factor is involved in the differential toxicities shown by the two herbicides.

If the differences in toxicity of the two grasses to simazine and atrazine are due to the greater persistence of simazine through retention of simazine by the soil organic and clay fractions, then these differences should disappear when the plants were grown in sand media (figures 3 and 4). Since in sand media the relative differences in toxicity of simazine and atrazine to the grasses remain, it is evident that differential soil adsorption of the two

herbicides is not responsible for the observed differences in toxicity.

Differences in uptake have been reported to be a factor in the selectivity of certain s-triazines on a limited number of plants (39). This is reflected in the use of simazine in the selective control of weeds in certain deep rooted crops. Simazine because of its low solubility and strong adsorption on to colloidal soil particles, is mostly restricted to the top 3 or 4 inches of the soil (16,39.63). Another possibility for differential uptake is that the resistant plant for some physiological reason, take up less herbicide (62). However, this phenomenon has not been noted with the s-triazines.

Differences in uptake of s-triazines have been noted between different plant species. Roeth and Lavy (71) found that ^{14}C-atrazine concentrations were two to three times greater in sorghum and sudangrass than in corn throughout a five week period. Davis et al. (24) studied the absorption of atrazine by corn, cotton and soybean plants. They found that soybean the most susceptible of the three species absorbed more atrazine per gram of fresh weight than did corn and cotton. In similar studies Thompson et al. (99) reported greater uptake of atrazine by corn than by oats, giant foxtail, fall panicum and large crabgrass. In other

work where the amount of herbicide taken up by resistant and susceptible plants have been compared little difference was found between the two types of plants (21,39.40). Differences in uptake patterns of herbicides have also been found to exist in some plants (51,71). Most researchers however have disregarded differences in uptake or in uptake patterns as contributing greatly to the wide differences in susceptibility of different plant species to the s-triazines.

In this investigation, uptake is not a factor contributing to differences in tolerance of crabgrass and witchgrass to atrazine and simazine (Table 1) as the amounts of ^{14}C activity found in the two plant species is not significantly different. Simazine and atrazine are taken up in similar quantities by crabgrass and witchgrass and differential absorption of herbicide does not account for the greater toxicity of simazine to the two plants. The pattern of uptake of simazine and atrazine are also similar (figure 5); the uptake of the s-triazine herbicides after 2,4,12 and 24 hours not differing significantly.

The extent of s-triazine degradation has been shown to be in good agreement with the relative susceptibilities of plants (21,72,78,100). However, the metabolic pathways of the s-triazines vary in different plants. Regardless

of the pathway, metabolism of the s-triazine herbicides results in total or partial detoxication.

Atrazine is metabolized at a faster rate in crabgrass than in witchgrass. If the chloroform-soluble metabolite found in the plants is unchanged s-triazine herbicide, then, one may account for the greater susceptibility of both plants to simazine on the basis of the quantities of unchanged s-triazine herbicide in the plant tissues. Also, smaller amounts of unchanged atrazine found in crabgrass could be responsible for the higher tolerance of crabgrass as compared to witchgrass to atrazine. However, since the amounts of chloroform-soluble simazine metabolite was similar for both plant species, differential metabolism could not possibly account for observed differences in simazine tolerance between crabgrass and witchgrass.

The importance of translocation on the tolerance of plants to the s-triazines have received only scant attention. Roeth and Lavy (71) found that the roots of corn contained larger percentages of total plant ^{14}C-atrazine than did the roots of sorghum or sudangrass, and suggested that longer retention of atrazine by corn roots would allow more time for metabolism before the herbicide was translocated to the shoots. Thompson *et al.* (99) demonstrated that corn translocated less ^{14}C-atrazine than did the more susceptible

oats, giant foxtail, fall panicum and large crabgrass. Although these differences were sizable, they considered them not large enough to contribute greatly to the wide differences in observed susceptibility to atrazine.

Bearing in mind that the s-triazine herbicides inhibit processes in photosynthetic tissue, the greater translocation of ^{14}C herbicides to the shoots by witchgrass cannot be ignored. In addition it may explain differences between witchgrass and crabgrass susceptibility to simazine which could not be explained on the basis of herbicide metabolism.

The peptide conjugation of chloro-s-triazines by Digitaria species is known to occur (99,100) but has not been reported previously for witchgrass. Shimabukuro et al. (91) demonstrated a recovery of photosynthetic oxygen evolution with the concomitant appearance of peptide conjugation of atrazine in corn leaf discs. Metabolism of the chloro-s-triazines to their corresponding peptide conjugates is therefore thought to be a detoxication mechanism. In this study the ability of each plant species to metabolize atrazine to peptide conjugates show a direct correlation with their resistance to the herbicide.

In other work (90) it was shown that the extent of peptide conjugate formation in corn was dependent on the activity of a soluble enzyme, glutathione-s-transferase.

They found a significant amount of unchanged atrazine accumulated in the susceptible corn line GT112 which showed low glutathione-s-transferase activity. In tolerant corn lines the enzymatic reaction predominated in the leaf blades where both glutathione-s-transferase and high concentrations of benzoxazinone existed. Benzoxazinone catalyzed hydroxylation was significant only when atrazine was introduced into the root tissue where little or no glutathione-s-transferase activity was found to exist.

The activity of glutathione-s-transferase in crabgrass and witchgrass seems to be in agreement with the observed differences in the ability of the two weedy grasses to metabolize atrazine. In vitro, the specific activity of glutathione-s-transferase for simazine and atrazine was such that atrazine was conjugated 12.5 times as fast as simazine (29). This low specificity of the enzyme for simazine accounts for the very slow metabolism of simazine by crabgrass and witchgrass, and for the apparent lack of differences observed in the amounts of hydrophilic metabolite(s) formed in the two grass species.

S U M M A R Y

Growth chamber studies showed crabgrass to be more tolerant than witchgrass to simazine and atrazine. Both grasses were more susceptible to simazine than to atrazine. Differences in toxicity of simazine and atrazine to the two grasses were not due to differential adsorption of the s-triazine herbicides by colloidal soil particles, as differences remained when the plants were treated with the herbicides in sand media.

Simazine and atrazine were taken up in similar quantities by the two plant species, thereby excluding the possibility that differential absorption of the herbicides accounted for the observed differences in susceptibility of crabgrass and witchgrass to the s-triazine herbicides.

The rate of atrazine metabolism differed in the two plant species, crabgrass being more effective than witchgrass in metabolizing atrazine. This phenomenon likely explains the greater tolerance of crabgrass to atrazine. Simazine was more toxic than atrazine to the grasses because it was more slowly metabolized by the plants. Metabolism of the herbicides apparently did not occur

in the roots of crabgrass and witchgrass as all the ^{14}C activity in these organs chromatographed as unchanged s-triazines.

Differences in tolerance to simazine between crabgrass and witchgrass are thought to be due to differences in their translocation rates. Witchgrass translocated greater quantities of ^{14}C s-triazines to the shoots (photosynthetic sites) than did crabgrass.

Both witchgrass and crabgrass converted the herbicides to their corresponding non-toxic peptide conjugates. This conversion is dependent on the presence of the enzyme glutathione-s-transferase. The ability of crabgrass and witchgrass to convert atrazine to its corresponding peptide conjugate was found to be related to the activity of glutathione-s-transferase in these plants.

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